

## Article

# Environmental Factors Shaping the Culturable Freshwater Fungi Diversity of Four Lakes in Yunnan Province, China

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**Citation:** Li, L.; Zhao, Z.-X.; Gui, H.; Wang, X.-A.; Xing, P.; Karunaratna, S.C.; Cheewangkoon, R. Environmental Factors Shaping the Culturable Freshwater Fungi Diversity of Four Lakes in Yunnan Province, China. *Diversity* **2024**, *16*, 612. <https://doi.org/10.3390/d16100612>

Academic Editor: Gaetan Burgaud

Received: 14 June 2024

Revised: 22 September 2024

Accepted: 23 September 2024

Published: 1 October 2024



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**Abstract:** Our study focused on freshwater fungal diversity, an important aspect in assessing the ecology of aquatic ecosystems. We carefully explored the diversity and influencing factors of culturable fungi across Dianchi Lake, Fuxian Lake, Xingyun Lake, and Yangzonghai Lake in Yunnan Province, China. Through fungi culture, morphological characterization, and ITS sequence analysis, we identified a total of 565 isolates belonging to 405 species across 133 genera. The diversity indices viz.  $H'$ ,  $D$ , and  $J$  were evaluated for fungal diversity across the lakes. Interestingly, although diversity indices were highest during summer and at Yangzonghai Lake, no significant differences in fungal community diversity were observed between seasons and regions. Water variables were analyzed and indicated that changes with rapid fluctuations in temperature, pH, and dissolved oxygen likely influence fungal diversity. These findings significantly contribute to our understanding of fungal communities within plateau lake ecosystems, thereby aiding in managing and conserving vital aquatic resources.

**Keywords:** aquatic fungi; diversity; ecology; lake ecosystems; plateau lake

## 1. Introduction

Yunnan, situated in southwestern China, is a region of extremes, ranging from the lofty heights of 6740 m in the northwest to the modest 76 m in the southeast, with an average elevation of about 2000 m above sea level. Its climate, a distinctive monsoon pattern, is characterized by an annual mean temperature of approximately 15 °C and an annual mean precipitation of about 1000 mm. The region is dotted with nine plateau lakes, namely Dianchi Lake, Erhai Lake, Fuxian Lake, Chenghai Lake, Lugu Lake, Qilu Lake, Yilong Lake, Xingyun Lake, and Yangzonghai Lake [1–3]. This unique blend of topographic complexity and favorable moisture conditions has a profound impact, fostering an environment that supports an astonishing richness of biological diversity and high degrees of endemism [4].

The significance of this diversity is underscored by numerous studies, with freshwater fungi, especially ascomycetes, emerging as a significant contributor.

Yunnan Province, a focal point of freshwater fungi biodiversity [3], has recently reported many new freshwater fungi. In particular, it has emerged as a hot spot for freshwater fungal research [3,5,6]. The streams and rivers of northwestern Yunnan have been the subject of rigorous investigation, a process that has led to the identification of numerous new species and novel records within diverse genera, such as *Acrogenospora*, *Dictyosporium*, *Distoseptispora*, *Pleurotheciella*, *Sporidesmium*, and *Sporoschisma* [3,5,7–15].

Freshwater fungi, a diverse and ecologically defined group of true fungi, are a crucial part of many species' life cycles, relying on freshwater for their entire or partial life cycle [16]. Calabon et al. reported that freshwater fungi occur across all fungal phyla, but predominantly within Ascomycota, Chytridiomycota, and Basidiomycota. The most specious phylum is the Ascomycota. Chytrids are crucial role in freshwater ecosystems, but their ecological importance is poorly understood. These ubiquitous organisms, found in a wide range of habitats including lentic (lakes, ponds) and lotic (rivers, streams, creeks, and peat swamps), are a mix of ascomycete anamorphic taxa and a few basidiomycetes [17–22]. Their importance cannot be overstated, as they play a vital role in material and energy recycling in freshwater ecosystems [23–26]. They possess the unique ability to degrade the cellulose and lignocellulose of woody debris in freshwater, forming a soft rot [27–29].

Recent studies have increasingly highlighted the multifaceted ecological roles of freshwater fungi, shedding light on their importance in aquatic ecosystems. For instance, Suberkropp (2011) demonstrated that freshwater fungi significantly influence the leaf litter and decomposition rates in streams, affecting nutrient release and cycling. Their study found that fungal-driven decomposition processes contribute to the overall nutrient dynamics and energy flow within stream ecosystems [30]. Gulis et al. explored how freshwater fungi modify woody debris in streams, enhancing its palatability for aquatic invertebrates. The study revealed that fungal degradation of wood increases its nutritional value for detritivores, thus impacting food web dynamics and invertebrate populations [31]. Mirabile et al. investigated the role of freshwater fungi in shaping stream habitats by decomposing submerged plant material. Their findings highlight how fungal activity creates microhabitats that support diverse aquatic communities, illustrating fungi's role in habitat complexity and biodiversity [32]. Gulis et al. showed that freshwater fungi influence water quality by decomposing organic matter and recycling nutrients. Their study emphasized the fungi's role in maintaining balanced nutrient levels and promoting overall ecosystem health [33].

In recent years, lake ecosystems have been severely affected by climate change and human activities, which may fundamentally alter the overall state of lakes [34–37]. As an important indicator of lake water quality, microorganisms influence the circulation of carbon, nitrogen, and phosphorus in lakes through their community structure, diversity, function, and activity [38]. They can reflect and assess the changes in the water environment to a certain extent.

Few reports investigate freshwater fungi in several lakes in Yunnan Province, China. However, they are all based only on morphology, and no studies are based on combined morphological, phylogenetic, and diversity analysis. Moreover, no systematic study of freshwater fungi has yet been conducted in Dianchi Lake, Fuxian Lake, Xingyun Lake, and Yangzonghai Lake of Yunnan Province, China. Dianchi Lake and Yangzonghai are geographically located in Kunming City, while Fuxian Lake and Xingyun Lake are located in Yuxi City. These two cities are the main cities in Yunnan Province, and Kunming City is the capital city of Yunnan Province [39]. The pollution levels in the four lakes vary, and we lack information on how different pollution levels affect the diversity of freshwater fungi. According to the 2022 environmental bulletin from the Yunnan Province Department of Ecology and Environment, the pollution levels are as follows: Dianchi Lake: V, Fuxian Lake: II, Xingyun Lake: V, and Yangzonghai Lake: II ([https://sthjt.yn.gov.cn/hjzl/hjzkgb/202306/t20230602\\_234194.html](https://sthjt.yn.gov.cn/hjzl/hjzkgb/202306/t20230602_234194.html), accessed on 1 May 2024). Based on the above reasons,

this paper aims to investigate: 1. the fungal diversity and distribution characteristics of the four lakes; 2. the effects of environmental factors on the culturable fungal diversity of the four lakes. In this study, while exploring the diversity of culturable fungi in the four lakes and the correlation between their physicochemical factors, the corresponding fungal isolates will also be obtained, which will provide strain resources and data support for the development and utilization of the fungal functions of the four lakes, as well as the impact of climate change on the microbial community structure of the plateau lakes.

2. Materials and Methods

2.1. Study Area and Experimental Design

Yunnan Province is located in the southwestern region of China. Its geographic location is 97°31′–106°11′ E, 21°8′–29°15′ N. This study selected the sampling sites of Dianchi Lake, Fuxian Lake, Xingyun Lake, and Yangzonghai Lake. See Table 1 for specific information. Since the water quality of Fuxian Lake is clear and there is less submerged wood on the lake, the sampling times were increased.

Table 1. Overview of sampling sites in four lakes of Yunnan Province, China.

| Sites            | Latitude      | Longitude       | Altitude (m) | Lake Area (km <sup>2</sup> ) | Collection Time  |
|------------------|---------------|-----------------|--------------|------------------------------|------------------|
| Dianchi Lake     | 24°22′–25°36′ | 102°22′–102°58′ | 1887.5       | 330                          | 13 April 2022    |
|                  |               |                 |              |                              | 26 July 2022     |
|                  |               |                 |              |                              | 13 October 2022  |
|                  |               |                 |              |                              | 21 April 2022    |
| Fuxian Lake      | 24°31′–24°51′ | 102°43′–102°59′ | 1720         | 212                          | 14 August 2022   |
|                  |               |                 |              |                              | 20 October 2022  |
|                  |               |                 |              |                              | 29 December 2022 |
|                  |               |                 |              |                              | 22 March 2023    |
| Xingyun Lake     | 24°33′        | 102°78′         | 1722         | 34.71                        | 1 July 2023      |
|                  |               |                 |              |                              | 21 April 2022    |
|                  |               |                 |              |                              | 13 August 2022   |
|                  |               |                 |              |                              | 20 October 2022  |
| Yangzonghai Lake | 24°51′–24°58′ | 102°5′–103°02′  | 1770         | 31.1                         | 10 March 2022    |
|                  |               |                 |              |                              | 12 July 2022     |
|                  |               |                 |              |                              | 18 October 2022  |

2.2. Sample Collection, Isolation, and Morphological Studies

Submerged decaying wood twigs and branches were collected from Yunnan Provinces, China. Fresh specimens were studied following the methods described by Luo et al. [12]. The samples were placed in plastic containers and incubated at room temperature for a week. Micromorphological characters were observed using a stereomicroscope (SteREO Discovery.V12, Carl Zeiss Microscopy GmbH, Oberkochen, Germany) and photographed using a Nikon ECLIPSE 80i (Tokyo, Japan) compound microscope fitted with a NikonDS-Ri2 digital camera (Tokyo, Japan). Microscopic structures were measured using the Tarosoft (R) Image Frame Work program v. 0.9.7. and the photomicrographs were processed using Adobe Photoshop CS6 version 10.0 software (Adobe Systems, San Jose, CA, USA).

Single spore isolation, a crucial step in our research, was performed following the method described by Luo et al. [12]. The germinated conidia were carefully transferred to fresh PDA plates and incubated at room temperature. The specimens were dried under natural light, wrapped in absorbent paper and placed in a Ziplock bag with mothballs. The herbarium specimens were deposited in the Herbarium of Cryptogams, Kunming Institute of Botany Academia Sinica (KUN-HKAS), Kunming, China. The cultures were deposited in Kunming Institute of Botany, Chinese Academy of Sciences (KUNCC), Kunming, Yunnan, China.

### 2.3. DNA Extraction, PCR Amplification and Sequencing

Fresh mycelia were scraped from colonies grown on potato dextrose agar (PDA) medium. DNA extraction was performed using the TOLOBIO Plant Genomic DNA Extraction Kit, Tsingke Company, Beijing, China, a kit known for its high quality and reliability. PCR amplification was performed using primer pairs ITS5/ITS4 [40] for the internal transcribed spacer rDNA region (ITS). The PCR amplification was carried out in a 25  $\mu$ L reaction volume containing 12.5  $\mu$ L of 2  $\times$  Power Taq PCR Master Mix, 1  $\mu$ L of each forward and reverse primer (10  $\mu$ M), 1  $\mu$ L of genomic DNA template (30–50 ng/ $\mu$ L) and 9.5  $\mu$ L of sterilized double-distilled water. Amplifications were carried out using the BioTeke GT9612 thermocycler (Tsingke Company, Beijing, China). The PCR amplification conditions for ITS consisted of initial denaturation at 98  $^{\circ}$ C for 3 min, followed by 35 cycles of denaturation at 98  $^{\circ}$ C for 20 s, annealing at 53  $^{\circ}$ C for 10 s, an extension at 72  $^{\circ}$ C for 20 s, and a final extension at 72  $^{\circ}$ C for 5 min. PCR product quality was checked using 1% agarose gel electrophoresis, and distinct bands were visualized in the gel documentation system (Compact Desktop UV Transilluminator analyzer GL-3120). Tsingke Company, Beijing, China purified and sequenced the PCR products, ensuring the reliability of the results.

### 2.4. Sequence Alignments and Phylogenetic Analyses

The newly generated sequences underwent a rigorous nucleotide BLAST search via NCBI (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>, accessed on 14 February 2024) to identify closely related taxa and ensure the accuracy of the sequences. All consensus sequences were meticulously submitted to the GenBank database under the accession numbers (PP620729–PP620794). Multiple sequence alignments were carefully aligned with MAFFT v.7 (<http://mafft.cbrc.jp/alignment/server/index.html>, accessed on 14 February 2024) and automatically trimmed using TrimAl (<http://phylemon.bioinfo.cipf.es/utilities.html>, accessed on 14 February 2024). A comprehensive combined sequence dataset was created with SquenceMatrix v.1.7.8 [41–43]. Phylogenetic relationships of the dominant genera were then established based on maximum likelihood (ML).

Maximum likelihood (ML) analysis was performed by RAxML-HPC2 v.8.2.12 on the XSEDE (8.2.12) tool via the CIPRES Science Gateway (<http://www.phylo.org/portal2>, accessed on 14 February 2024) [44,45]. The analysis was followed the default setting but adjusted by setting 1000 bootstrap replications and the GTRGAMMA model of nucleotide substitution. Phylograms were visualized using FigTree v1.4.0 [46] and rearranged in Adobe Photoshop CS6 software (Adobe Systems, USA).

Based on freshwater fungi's morphological classification and molecular identification results, a thorough analysis was conducted on the obtained data. The percentage of isolates of the same genus or species to all isolates is  $\geq 1.5\%$ , indicating the dominance of a particular genus or species; 1–1.5% are common genera or species;  $\leq 1\%$  are rare genera or species. These findings are crucial in understanding the biodiversity and ecological roles of freshwater fungi.

### 2.5. Measurement of Physical and Chemical Indicators of Water

The lake water temperature, pH, dissolved oxygen (DO), electrical conductivity (EC), and other physical and chemical indexes were measured by a portable three-channel analyzer (YSI HQ40d, Loveland, CO, USA) at the sampling site. A turbidimeter measured the turbidity of water. Water samples around the sampling site were taken from the water environment and stored at a low temperature. After returning to the laboratory, some of the water samples were placed under a microscope, and the algal density was calculated based on the number of cells per unit volume. The filter was filtered through 0.45  $\mu$ m glass fiber membrane filter (MerckMillipore Ltd., Ireland), ammonia ( $\text{NH}_4^+$ ) analysis was determined by Natchellott reagent colorimetric method, nitrate ( $\text{NO}_3^-$ ) was determined by ultraviolet spectrophotometry (Auto Analyzer III, Seal, Germany), total phosphorus (TP) was determined by ammonium molybdate spectrophotometry (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China), and total nitrogen (TN) was determined by ultraviolet

spectrophotometry after alkaline potassium persulfate digestion (Beijing Chemical Works, Beijing, China). The use of different methods for different types of analysis, such as colorimetric and spectrophotometric, demonstrated the thoroughness of the research and the variety of factors considered in water quality assessment. The chemical oxygen demand (COD) in the water body was determined by acid high-oxide solution (such as potassium sodium sulfate solution) according to GB3838-2002 (Environmental Quality Standards for Surface Water. National Standards of the People's Republic of China) and soaked in 90% acetone as extraction reagent (ASTMD3731-87) (Standard Test Method for Determination of Chlorophyll in Water) at 4 °C after filtration and grinding, and then the chlorophyll content (CHA) was measured by ultraviolet spectrophotometer [47,48].

## 2.6. Data Analysis

The Plotly package of Python (v3. 11. 8) was employed for visualization [49]. For the fungal community's analysis, the non-metric multidimensional scaling (NMDS) analysis was performed using the Scikit-learn package of Python [50]. To determine the distribution of fungal characteristics, the Venn and upsetplot of Python were utilized [51]. The Mantel analysis was performed using Vegan [49]. For the Co-occurrence network analysis of the environment factor to the fungi, the SciPy package of Python was used for the statistical difference and correlation analysis ( $p < 0.05$ ,  $R > 0.7$ ) [52], and the networkx package of Python was employed. The random forest analysis was performed using the Scikit-learn package of Python [53]. In Excel 2016, calculate the Shannon–Wiener Diversity Index ( $H'$ ), Pielou Evenness Index ( $J$ ), Simpson Diversity Index ( $D$ ), Occurrence Frequency (OF), and Relative Abundance (RA). The resulting output was performed using ggplot2 [54].

The formulas for diversity are as follows:

$$H' = -\sum P_i \ln P_i \quad (1)$$

$$J = H' / \ln(N) \quad (2)$$

$$D = 1 - \sum P_i^2 \quad (3)$$

$$OF = (\text{The number of sample points that a strain occurrences} / \text{total sample points}) \times 100\% \quad (4)$$

$$RA = (\text{Total number of a strain} / \text{Total number of all isolates}) \times 100\% \quad (5)$$

The non-metric multidimensional scaling (NMDS) analysis was conducted using the Scikit-learn package in Python. Prior to analysis, the data were transformed using the “wisconsin” method, followed by the calculation of Bray–Curtis distances. The optimal configuration was selected based on the lowest stress values. The final stress values for the NMDS analysis were 0.0461 for spatial distribution and 0.109 for seasonal distribution, indicating a good fit for both dimensions.

The NMDS analysis is as follows:

The data to be analyzed were a collection of  $M$  objects (season, study area) on which a distance function was defined, and  $X_{i,j}$  was the abundance of the  $i$ -species and the  $j$ -sample in  $D$  objects.

$$\mathbf{M} = \begin{bmatrix} M_{S1} & M_{T1} \\ M_{S2} & M_{T1} \\ \vdots & \vdots \\ M_{Si} & M_{Ti} \end{bmatrix} \quad \mathbf{D} = \begin{bmatrix} X_{1,1} & X_{1,2} & \cdots & X_{1,j} \\ X_{2,1} & X_{2,2} & \cdots & X_{2,j} \\ \vdots & \vdots & \ddots & \vdots \\ X_{i,1} & X_{i,2} & \cdots & X_{i,j} \end{bmatrix}$$

The dissimilarity matrix  $D$  will be of size  $M \times M$ . The species abundance matrix can be constructed using a variety of distance metrics of Bray–Curtis distance.

$$d_{i,j} = \frac{\sum_{k=1}^p |X_{ik} - X_{jk}|}{\sum_{k=1}^p (X_{ik} + X_{jk})}$$

$d_{ij}$  is the Bray–Curtis distance between sample  $i$  and sample  $j$ .  
 $X_{i,k}$  and  $X_{j,k}$  are the abundances of species  $k$  in samples  $i$  and  $j$ , respectively.  
 $p$  is the total number of species.

These distances are the entries of the dissimilarity matrix  $D$  as follow:

$$D = \begin{bmatrix} 0 & d_{1,2} & d_{1,3} & \cdots & d_{1,j} \\ d_{2,1} & 0 & d_{2,3} & \cdots & d_{2,j} \\ d_{3,1} & d_{3,2} & 0 & \cdots & d_{3,j} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ d_{i,1} & d_{i,2} & d_{i,3} & \cdots & 0 \end{bmatrix}$$

Once the samples are positioned, calculate the pairwise Euclidean distances in this low-dimensional space. The distance between samples  $i$  and  $j$  is:

$$y_{ij} = \sqrt{\sum_{m=1}^k (y_{i,m} - y_{j,m})^2}$$

$k$  is the dimension of the low-dimensional space (e.g., 2D, where  $k = 2$ ).

$y_{i,m}$  and  $y_{j,m}$  are the coordinates of samples  $i$  and  $j$  in the low-dimensional space.

The goal of NMDS is to adjust the positions of the samples in the low-dimensional space so that the distances  $y_{ij}$  resemble the original distances  $d_{ij}$  as much as possible. This is achieved by minimizing the stress function:

$$\text{Stress} = \sqrt{\frac{\sum_{i < j} (f(d_{ij}) - y_{ij})^2}{\sum_{i < j} y_{ij}^2}}$$

$f(d_{ij})$  is a monotonic transformation of the original Bray–Curtis distances (since NMDS focuses on rank order rather than absolute values).

The NMDS algorithm iteratively adjusts the positions of the samples, recomputes the stress, and repeats the process until the stress value converges to a sufficiently low level or the maximum number of iterations is reached.

Choose the main columns 1 and 2, and finally obtain the species distance matrix as follows:

$$MD = \begin{bmatrix} M_{S1} & M_{T1} & d_{1,1} & d_{1,2} \\ M_{S2} & M_{T2} & d_{2,1} & d_{2,2} \\ \vdots & \vdots & \vdots & \vdots \\ M_{Si} & M_{Ti} & d_{i,1} & d_{i,2} \end{bmatrix}$$

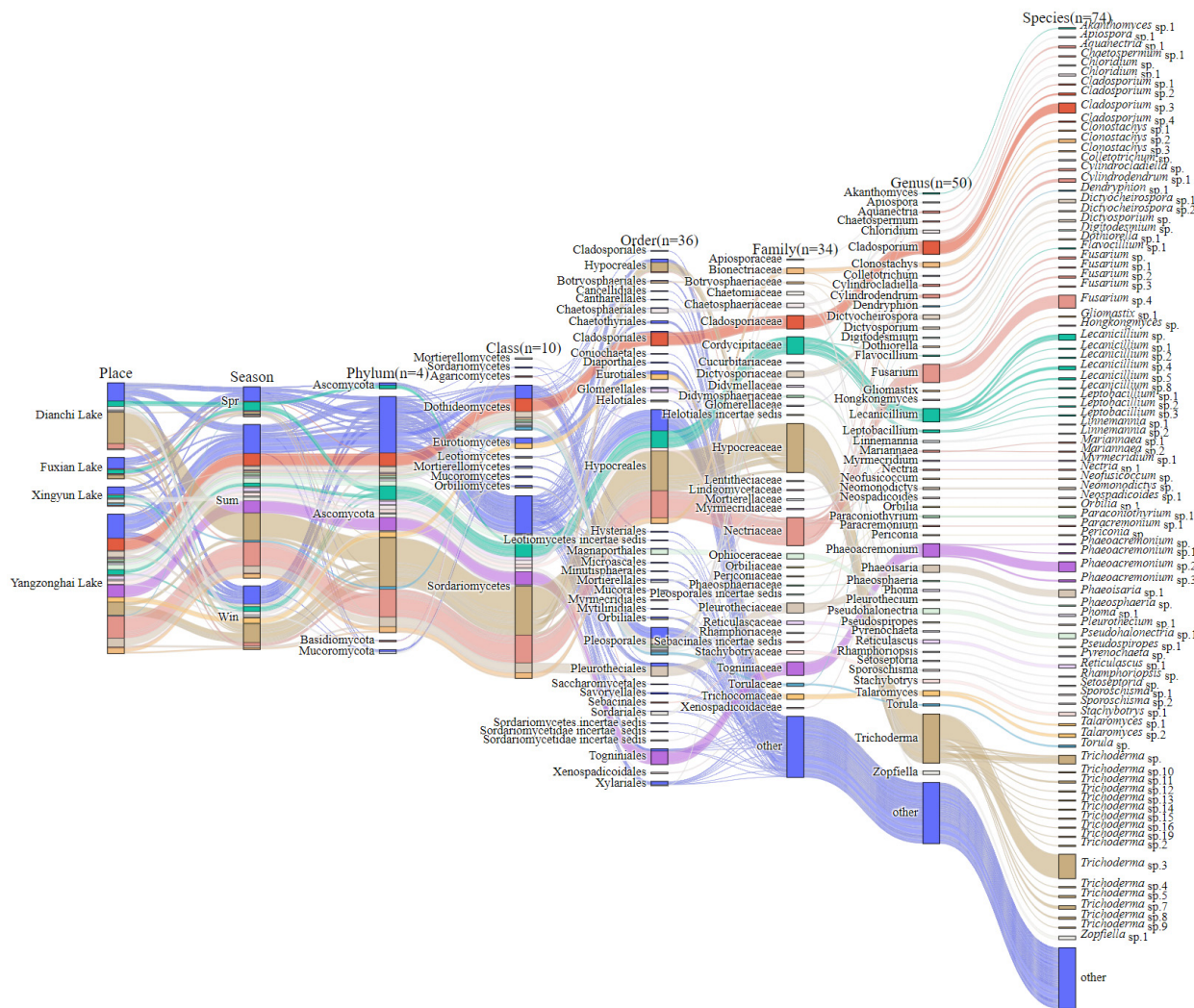
### 3. Results

#### 3.1. Culturable Freshwater Fungal Community Composition

Distribution of Culturable Freshwater Fungi Genera and Species in Four Lakes in Yunnan Province, China

A total of 565 cultivable freshwater fungi were isolated and purified across the four lakes. After morphological and phylogenetic analysis, they were identified as 133 genera (Table 2 and Figure 1) and 405 species, with 113 in Dianchi Lake, 63 in Fuxian Lake, 47 in Xingyun Lake, and 182 in Yangzonghai Lake. By contrast, in the distribution of different seasons, the most species of cultivable freshwater fungi were in summer ( $n = 193$ ), followed by winter ( $n = 129$ ) and spring ( $n = 83$ ). Distribution of culturable freshwater fungi genera in four lakes of Yunnan Province (Table 2 and Figure 1) displayed *Dictyocheiropsora*, *Fusarium*, *Lecanicillium*, *Neopyrenochaeta*, *Periconia*, *Penicillium*, *Phaeoacremonium*, *Trichoderma* and had the highest detected frequency of occurrence, which was 100%, while 15 genera had the frequency of occurrence of 75%, 23 genera had the frequency of occurrence of 50%, and 87 genera had the lowest occurrence rate of 25%. Furthermore, the total abundance of culturable freshwater fungi at the species level was illustrated in Figure 1, while the species

with a relative abundance of less than 1% were attributed to “other”. There were 331 rare species, accounting for about 81% of the total species and 55.1% of the total abundance. This indicated that the water fungi communities were predominantly dominated by a small number of taxa (Figure 1). As shown in Figure 1, *Trichoderma*, *Lecanicillium*, and *Fusarium* were the three most reported genera, accounting for 9.5%, 3.8%, and 3.1% of the total abundance. Among the species, *Trichoderma* sp.10, *Fusarium* sp.1, and *Phaeoisaria* sp. 2 were the three most reported species than any other species, accounting for 3.3%, 2.3%, and 1.5% (Table 3).



**Figure 1.** Total abundance of culturable freshwater fungi at the species level. Note: Species with a relative abundance less than 1% are denoted as others.

**Table 2.** Distribution of culturable freshwater fungi genera in four lakes of Yunnan Province.

| Genus                | Dianchi Lake | Fuxian Lake | Xingyun Lake | Yangzonghai Lake | OF (%) | RA (%) |
|----------------------|--------------|-------------|--------------|------------------|--------|--------|
| <i>Acremonium</i>    | 1            | 0           | 0            | 0                | 25     | 0.18   |
| <i>Acrogenospora</i> | 1            | 0           | 0            | 0                | 25     | 0.18   |
| <i>Akanthomyces</i>  | 0            | 0           | 0            | 3                | 25     | 0.53   |
| <i>Alternaria</i>    | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Apiospora</i>     | 6            | 0           | 3            | 1                | 75     | 1.77   |
| <i>Aquanectria</i>   | 1            | 0           | 0            | 3                | 50     | 0.71   |

Table 2. Cont.

| Genus                    | Dianchi Lake | Fuxian Lake | Xingyun Lake | Yangzonghai Lake | OF (%) | RA (%) |
|--------------------------|--------------|-------------|--------------|------------------|--------|--------|
| <i>Arthrinium</i>        | 1            | 1           | 0            | 0                | 50     | 0.35   |
| <i>Arthrobotrys</i>      | 1            | 1           | 0            | 1                | 75     | 0.53   |
| <i>Aspergillus</i>       | 0            | 0           | 0            | 3                | 25     | 0.53   |
| <i>Atractium</i>         | 0            | 0           | 0            | 2                | 25     | 0.35   |
| <i>Bipolaris</i>         | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Botryosphaeria</i>    | 0            | 1           | 0            | 0                | 25     | 0.18   |
| <i>Botrytis</i>          | 0            | 1           | 0            | 0                | 25     | 0.18   |
| <i>Cancellidium</i>      | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Capronia</i>          | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Cephalosporium</i>    | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Cephalotrichum</i>    | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Chaetomium</i>        | 0            | 1           | 0            | 0                | 25     | 0.18   |
| <i>Chaetosphaeria</i>    | 1            | 0           | 0            | 0                | 25     | 0.18   |
| <i>Chloridium</i>        | 6            | 0           | 0            | 0                | 25     | 1.06   |
| <i>Circinella</i>        | 3            | 0           | 0            | 0                | 25     | 0.53   |
| <i>Cladosporium</i>      | 0            | 3           | 2            | 15               | 75     | 3.54   |
| <i>Clohesyomyces</i>     | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Clonostachys</i>      | 1            | 0           | 1            | 14               | 75     | 2.83   |
| <i>Colletotrichum</i>    | 2            | 0           | 0            | 2                | 50     | 0.71   |
| <i>Coniochaeta</i>       | 0            | 1           | 0            | 0                | 25     | 0.18   |
| <i>Cordyceps</i>         | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Cosmospora</i>        | 2            | 0           | 0            | 0                | 25     | 0.35   |
| <i>Cylindrocladiella</i> | 1            | 0           | 0            | 3                | 50     | 0.71   |
| <i>Cylindrocladium</i>   | 0            | 1           | 0            | 0                | 25     | 0.18   |
| <i>Cylindrodendrum</i>   | 0            | 0           | 0            | 4                | 25     | 0.71   |
| <i>Dactylium</i>         | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Dactylonectria</i>    | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Dematiosporium</i>    | 2            | 0           | 1            | 0                | 50     | 0.53   |
| <i>Dendryphion</i>       | 0            | 0           | 0            | 3                | 25     | 0.53   |
| <i>Diaporthe</i>         | 0            | 1           | 0            | 1                | 50     | 0.35   |
| <i>Dictyocheiropora</i>  | 1            | 1           | 1            | 7                | 100    | 1.77   |
| <i>Dictyocheiropora</i>  | 0            | 1           | 0            | 0                | 25     | 0.18   |
| <i>Dictyosporium</i>     | 0            | 0           | 0            | 5                | 25     | 0.88   |
| <i>Didymella</i>         | 0            | 3           | 0            | 4                | 50     | 1.24   |
| <i>Didymellaceae</i>     | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Digitodesmium</i>     | 0            | 0           | 2            | 0                | 25     | 0.35   |
| <i>Distoseptispora</i>   | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Dothiorella</i>       | 0            | 0           | 2            | 1                | 50     | 0.53   |
| <i>Entoleuca</i>         | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Epicoccum</i>         | 0            | 1           | 0            | 1                | 50     | 0.35   |
| <i>Exophiala</i>         | 1            | 0           | 2            | 3                | 75     | 1.06   |
| <i>Flavocillium</i>      | 3            | 1           | 0            | 1                | 75     | 0.88   |
| <i>Fusarium</i>          | 10           | 3           | 4            | 22               | 100    | 6.9    |
| <i>Fusicolla</i>         | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Galactomyces</i>      | 0            | 1           | 0            | 0                | 25     | 0.18   |
| <i>Geosmithia</i>        | 0            | 1           | 0            | 0                | 25     | 0.18   |
| <i>Gliomastix</i>        | 1            | 0           | 0            | 3                | 50     | 0.71   |
| <i>Graphium</i>          | 0            | 1           | 0            | 0                | 25     | 0.18   |
| <i>Halobyssothecium</i>  | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Helminthosporium</i>  | 1            | 0           | 0            | 0                | 25     | 0.18   |
| <i>Hongkongmyces</i>     | 0            | 0           | 0            | 2                | 25     | 0.35   |
| <i>Hyalorbilia</i>       | 1            | 0           | 0            | 0                | 25     | 0.18   |
| <i>Hydropisphaera</i>    | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Idriella</i>          | 1            | 0           | 0            | 0                | 25     | 0.18   |
| <i>Juxtiphoma</i>        | 1            | 0           | 0            | 0                | 25     | 0.18   |
| <i>Lecanicillium</i>     | 9            | 7           | 5            | 10               | 100    | 5.49   |
| <i>Lentithecium</i>      | 0            | 0           | 1            | 1                | 50     | 0.35   |
| <i>Leptobacillium</i>    | 4            | 0           | 3            | 4                | 75     | 1.95   |
| <i>Linnemannia</i>       | 3            | 0           | 0            | 2                | 50     | 0.88   |

Table 2. Cont.

| Genus                         | Dianchi Lake | Fuxian Lake | Xingyun Lake | Yangzonghai Lake | OF (%) | RA (%) |
|-------------------------------|--------------|-------------|--------------|------------------|--------|--------|
| <i>Lophiostoma</i>            | 0            | 1           | 0            | 0                | 25     | 0.18   |
| <i>Mariannaea</i>             | 8            | 0           | 2            | 3                | 75     | 2.3    |
| <i>Memnoniella</i>            | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Minimelanolocus</i>        | 0            | 0           | 1            | 0                | 25     | 0.18   |
| <i>Montagnula</i>             | 0            | 1           | 0            | 0                | 25     | 0.18   |
| <i>Mortierella</i>            | 1            | 0           | 0            | 2                | 50     | 0.53   |
| <i>Mucor</i>                  | 2            | 0           | 0            | 0                | 25     | 0.35   |
| <i>Myrmecridium</i>           | 0            | 0           | 0            | 2                | 25     | 0.35   |
| <i>Mytilinidion</i>           | 1            | 0           | 0            | 0                | 25     | 0.18   |
| <i>Myxotrichum</i>            | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Nectria</i>                | 4            | 0           | 0            | 0                | 25     | 0.71   |
| <i>Nectriopsis</i>            | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Neofusicoccum</i>          | 0            | 0           | 0            | 3                | 25     | 0.53   |
| <i>Neomonodictys</i>          | 0            | 0           | 0            | 4                | 25     | 0.71   |
| <i>Neomultiseptospora</i>     | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Neopestalotiopsis</i>      | 1            | 0           | 0            | 1                | 25     | 0.35   |
| <i>Neopyrenochaeta</i>        | 1            | 1           | 1            | 1                | 100    | 0.71   |
| <i>Neospadicoides</i>         | 2            | 0           | 0            | 0                | 25     | 0.35   |
| <i>Nigrospora</i>             | 0            | 1           | 0            | 0                | 25     | 0.18   |
| <i>Orbilina</i>               | 1            | 2           | 0            | 1                | 75     | 0.71   |
| <i>Paracamarosporium</i>      | 1            | 0           | 0            | 1                | 50     | 0.35   |
| <i>Paraconiothyrium</i>       | 0            | 1           | 0            | 4                | 50     | 0.88   |
| <i>Paracremonium</i>          | 1            | 0           | 3            | 3                | 75     | 1.24   |
| <i>Penicillium</i>            | 2            | 2           | 1            | 3                | 100    | 1.42   |
| <i>Periconia</i>              | 1            | 1           | 1            | 3                | 100    | 1.06   |
| <i>Pestalotiopsis</i>         | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Phaeoacremonium</i>        | 6            | 4           | 1            | 14               | 100    | 4.42   |
| <i>Phaeoisaria</i>            | 2            | 1           | 0            | 9                | 75     | 2.12   |
| <i>Phaeosphaeria</i>          | 0            | 0           | 0            | 2                | 25     | 0.35   |
| <i>Phoma</i>                  | 1            | 2           | 0            | 3                | 75     | 1.06   |
| <i>Phomopsis</i>              | 1            | 0           | 0            | 0                | 25     | 0.18   |
| <i>Plectosphaerella</i>       | 0            | 1           | 0            | 0                | 25     | 0.18   |
| <i>Plenodomus</i>             | 1            | 1           | 0            | 0                | 25     | 0.35   |
| <i>Pleurotheciella</i>        | 0            | 1           | 1            | 3                | 75     | 0.88   |
| <i>Pleurothecium</i>          | 3            | 0           | 0            | 0                | 25     | 0.53   |
| <i>Podila</i>                 | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Pseudoastrosphaeriella</i> | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Pseudohalonectria</i>      | 0            | 0           | 1            | 7                | 50     | 1.42   |
| <i>Pseudorobillarda</i>       | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Pseudospiropes</i>         | 0            | 0           | 0            | 2                | 25     | 0.35   |
| <i>Purpureocillium</i>        | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Pyrenochaeta</i>           | 0            | 0           | 0            | 2                | 25     | 0.35   |
| <i>Pyrenochaetopsis</i>       | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Reticulascus</i>           | 0            | 0           | 0            | 4                | 25     | 0.71   |
| <i>Rhamphoriopsis</i>         | 2            | 0           | 0            | 0                | 25     | 0.35   |
| <i>Rhinochadiella</i>         | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Rhytidhysterium</i>        | 1            | 0           | 0            | 1                | 50     | 0.35   |
| <i>Samsoniella</i>            | 0            | 1           | 0            | 0                | 25     | 0.18   |
| <i>Savoryella</i>             | 0            | 1           | 1            | 1                | 75     | 0.53   |
| <i>Setoseptoria</i>           | 0            | 0           | 0            | 2                | 25     | 0.35   |
| <i>Simplicillium</i>          | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Sporoschisma</i>           | 3            | 0           | 0            | 2                | 50     | 0.88   |
| <i>Stachybotrys</i>           | 0            | 3           | 0            | 5                | 50     | 1.42   |
| <i>Stephanonectria</i>        | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Sterigmatobotrys</i>       | 1            | 1           | 1            | 0                | 75     | 0.53   |
| <i>Striatibotrys</i>          | 1            | 0           | 0            | 0                | 25     | 0.18   |
| <i>Talaromyces</i>            | 0            | 1           | 1            | 10               | 75     | 2.12   |
| <i>Thelonectria</i>           | 1            | 0           | 0            | 0                | 25     | 0.18   |
| <i>Thyridium</i>              | 0            | 0           | 0            | 1                | 25     | 0.18   |

Table 2. Cont.

| Genus                       | Dianchi Lake | Fuxian Lake | Xingyun Lake | Yangzonghai Lake | OF (%) | RA (%) |
|-----------------------------|--------------|-------------|--------------|------------------|--------|--------|
| <i>Torula</i>               | 0            | 2           | 4            | 0                | 50     | 1.06   |
| <i>Trematophoma</i>         | 0            | 0           | 0            | 1                | 25     | 0.18   |
| <i>Trichoderma</i>          | 33           | 13          | 7            | 29               | 100    | 14.51  |
| <i>Varicosporellopsis</i>   | 1            | 0           | 1            | 0                | 50     | 0.35   |
| <i>Veronaea</i>             | 1            | 0           | 1            | 0                | 50     | 0.35   |
| <i>Verticillium</i> ’ clade | 0            | 1           | 0            | 1                | 50     | 0.35   |
| <i>Zopfiella</i>            | 0            | 0           | 5            | 0                | 25     | 0.88   |
| unclassified                | 1            | 0           | 0            | 0                | 25     | 0.18   |
| <i>Hyaloscyphaceae</i>      |              |             |              |                  |        |        |
| <i>Xenoacremonium</i>       | 1            | 0           | 0            | 0                | 25     | 0.18   |
| TA(cfu/L)                   | 151          | 74          | 60           | 280              |        |        |

Table 3. Fungal taxa obtained from four lakes of Yunnan Province, identified by ITS sequence comparison with the BLATn match with NCBI GenBank database.

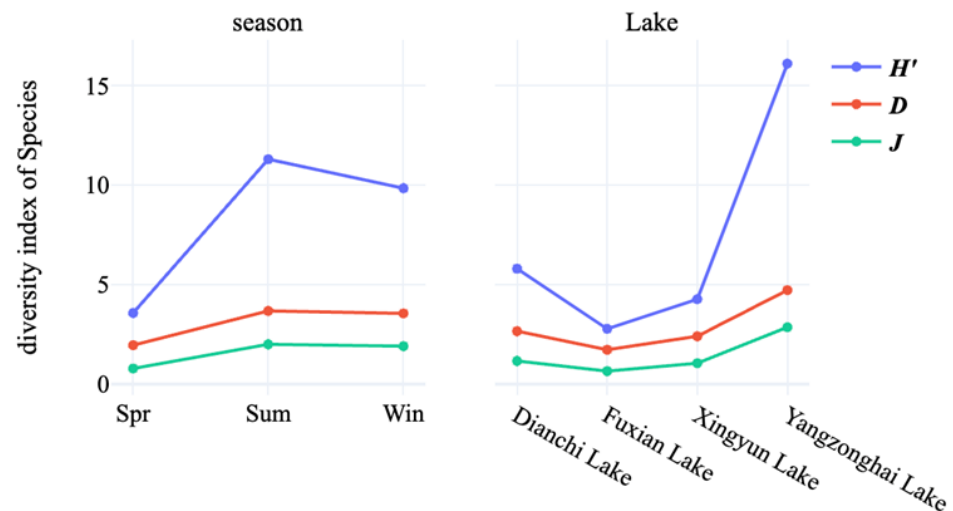
| Culture Number | GenBank Number | Blast Result                  |              |
|----------------|----------------|-------------------------------|--------------|
|                |                | Species Name                  | Identity (%) |
| KUNCC23-13242  | PP620729       | <i>Apiospora</i> sp.1         | 99.09%       |
| KUNCC23-13240  | PP620730       | <i>Apiospora</i> sp.2         | 100%         |
| KUNCC23-13224  | PP620731       | <i>Apiospora</i> sp.3         | 100%         |
| KUNCC22-12641  | PP620732       | <i>Apiospora</i> sp.          | 100%         |
| KUNCC23-13007  | PP620733       | <i>Apiospora</i> sp.4         | 97.21%       |
| KUNCC23-13264  | PP620734       | <i>Cladosporium</i> sp.1      | 99.80%       |
| KUNCC23-12949  | PP620735       | <i>Cladosporium</i> sp.2      | 100%         |
| KUNCC22-12617  | PP620736       | <i>Cladosporium</i> sp.3      | 100%         |
| KUNCC23-13256  | PP620737       | <i>Cladosporium</i> sp.4      | 100%         |
| KUNCC23-13678  | PP620738       | <i>Cladosporium</i> sp.5      | 100%         |
| KUNCC22-12559  | PP620739       | <i>Cladosporium</i> sp.       | 100%         |
| KUNCC23-13198  | PP620740       | <i>Cladosporium</i> sp.6      | 100%         |
| KUNCC22-12584  | PP620741       | <i>Cladosporium</i> sp.7      | 100%         |
| KUNCC22-12605  | PP620742       | <i>Clonostachys</i> sp.1      | 100%         |
| KUNCC23-12936  | PP620743       | <i>Clonostachys</i> sp.2      | 100%         |
| KUNCC23-12897  | PP620744       | <i>Clonostachys</i> sp.       | 100%         |
| KUNCC22-12575  | PP620745       | <i>Dictyocheiropsora</i> sp.1 | 100%         |
| KUNCC23-13639  | PP620746       | <i>Dictyocheiropsora</i> sp.2 | 99.81%       |
| KUNCC23-16675  | PP620747       | <i>Dictyocheiropsora</i> sp.3 | 100%         |
| KUNCC22-12506  | PP620748       | <i>Fusarium</i> sp.7          | 100%         |
| KUNCC23-12721  | PP620749       | <i>Fusarium</i> sp.6          | 100%         |
| KUNCC23-14455  | PP620750       | <i>Fusarium</i> sp.5          | 100%         |
| KUNCC23-13216  | PP620751       | <i>Fusarium</i> sp.4          | 100%         |
| KUNCC23-12923  | PP620752       | <i>Fusarium</i> sp.3          | 99.81%       |
| KUNCC23-14545  | PP620753       | <i>Fusarium</i> sp.2          | 100%         |
| KUNCC22-12579  | PP620754       | <i>Fusarium</i> sp.1          | 100%         |
| KUNCC22-12583  | PP620755       | <i>Fusarium</i> sp.           | 99.24%       |

Table 3. Cont.

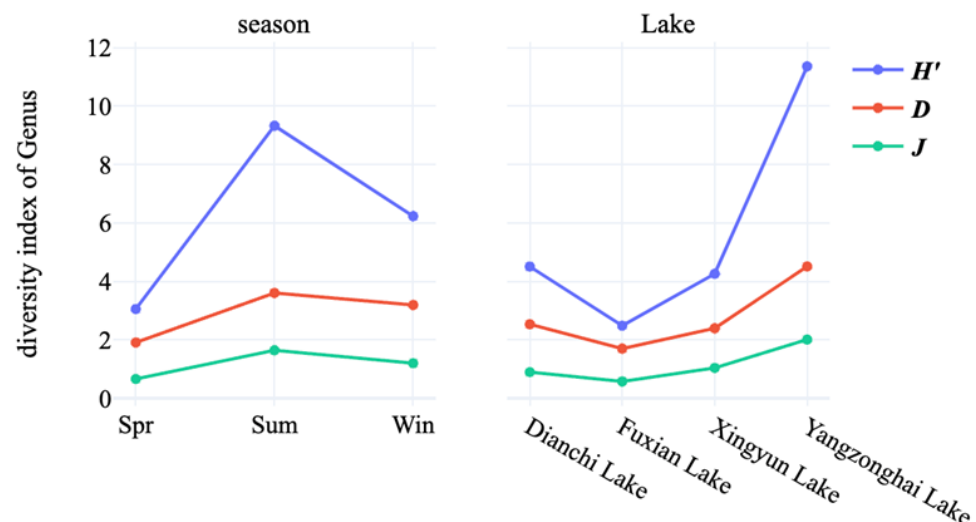
| Culture Number | GenBank Number | Blast Result                |              |
|----------------|----------------|-----------------------------|--------------|
|                |                | Species Name                | Identity (%) |
| KUNCC23-12960  | PP620756       | <i>Lecanicillium</i> sp.1   | 100%         |
| KUNCC22-12552  | PP620757       | <i>Lecanicillium</i> sp.2   | 99.64%       |
| KUNCC22-12486  | PP620758       | <i>Lecanicillium</i> sp.3   | 100%         |
| KUNCC22-12491  | PP620759       | <i>Lecanicillium</i> sp.    | 100%         |
| KUNCC22-12478  | PP620760       | <i>Leptobacillium</i> sp.1  | 99%          |
| KUNCC23-13273  | PP620761       | <i>Leptobacillium</i> sp.2  | 100%         |
| KUNCC23-12959  | PP620762       | <i>Mariannaea</i> sp.1      | 100%         |
| KUNCC22-12581  | PP620763       | <i>Mariannaea</i> sp.2      | 100%         |
| KUNCC23-13280  | PP620764       | <i>Mariannaea</i> sp.3      | 99.81%       |
| KUNCC22-12571  | PP620765       | <i>Mariannaea</i> sp.4      | 99.81%       |
| KUNCC23-13186  | PP620766       | <i>Phaeoacremonium</i> sp.1 | 99.57%       |
| KUNCC23-13636  | PP620767       | <i>Phaeoacremonium</i> sp.2 | 100%         |
| KUNCC23-14496  | PP620768       | <i>Phaeoacremonium</i> sp.3 | 99.44%       |
| KUNCC23-13666  | PP620769       | <i>Phaeoacremonium</i> sp.4 | 99.27%       |
| KUNCC23-13208  | PP620770       | <i>Phaeoacremonium</i> sp.5 | 99.64%       |
| KUNCC22-12502  | PP620771       | <i>Phaeoacremonium</i> sp.6 | 99.82%       |
| KUNCC23-12925  | PP620772       | <i>Phaeoacremonium</i> sp.  | 99.28%       |
| KUNCC23-12900  | PP620773       | <i>Phaeoisaria</i> sp.1     | 97.58%       |
| KUNCC22-12546  | PP620774       | <i>Phaeoisaria</i> sp.2     | 100%         |
| KUNCC22-12501  | PP620775       | <i>Talaromyces</i> sp.1     | 100%         |
| KUNCC23-14471  | PP620776       | <i>Talaromyces</i> sp.2     | 100%         |
| KUNCC23-14506  | PP620777       | <i>Talaromyces</i> sp.3     | 100%         |
| KUNCC23-14476  | PP620778       | <i>Talaromyces</i> sp.      | 99.44%       |
| KUNCC23-12883  | PP620779       | <i>Talaromyces</i> sp.4     | 100%         |
| KUNCC23-14456  | PP620780       | <i>Trichoderma</i> sp.1     | 100%         |
| KUNCC23-13215  | PP620781       | <i>Trichoderma</i> sp.2     | 100%         |
| KUNCC23-13245  | PP620782       | <i>Trichoderma</i> sp.3     | 100%         |
| KUNCC23-13344  | PP620783       | <i>Trichoderma</i> sp.4     | 99.82%       |
| KUNCC23-13606  | PP620784       | <i>Trichoderma</i> sp.5     | 100%         |
| KUNCC22-12488  | PP620785       | <i>Trichoderma</i> sp.6     | 100%         |
| KUNCC23-13617  | PP620786       | <i>Trichoderma</i> sp.7     | 100%         |
| KUNCC23-13608  | PP620787       | <i>Trichoderma</i> sp.9     | 100%         |
| KUNCC23-13181  | PP620788       | <i>Trichoderma</i> sp.10    | 100%         |
| KUNCC23-12888  | PP620789       | <i>Trichoderma</i> sp.11    | 100%         |
| KUNCC23-13610  | PP620790       | <i>Trichoderma</i> sp.12    | 99.81%       |
| KUNCC22-12614  | PP620791       | <i>Trichoderma</i> sp.      | 100%         |
| KUNCC22-12555  | PP620792       | <i>Trichoderma</i> sp.13    | 100%         |
| KUNCC23-12720  | PP620793       | <i>Trichoderma</i> sp.14    | 100%         |
| KUNCC23-13283  | PP620794       | <i>Trichoderma</i> sp.15    | 100%         |

### 3.2. Diversity Analysis of Cultivable Freshwater Fungal Species in Four Lakes

Analysis of the genera and species diversity index of culturable fungi in four lakes are shown in Figures 2 and 3, and Tables 4 and 5. From a seasonal perspective, the diversity index was highest in summer, followed by winter, the lowest in spring. The fungal diversity of the lakes was seen as Yangzonghai Lake > Dianchi Lake > Xingyun Lake > Fuxian Lake. The diversity index  $H'$  fluctuated greatly, while  $J$  and  $D$  fluctuated relatively little. At the species level, the data were as follows—Shannon index: summer (3.67) > winter (3.55) > spring (1.95), Yangzonghai Lake (4.71) > Dianchi Lake (2.65) > Xingyun Lake (2.4) > Fuxian Lake (1.72); Pielou index: summer (11.3) > winter (9.84) > spring (3.57), Yangzonghai Lake (16.1) > Dianchi Lake (5.79) > Xingyun Lake (4.26) > Fuxian lake (2.77); Simpson index: summer (1.99) > winter (1.9) > spring (0.77), Yangzonghai Lake (2.85) > Dianchi Lake (1.15) > Xingyun Lake (1.04) > Fuxian Lake (0.64).



**Figure 2.** Diversity indexes of freshwater fungi species;  $H'$ , Shannon index;  $J$ , Pielou index;  $D$ , Simpson index.



**Figure 3.** Diversity indexes of freshwater fungi genera;  $H'$ , Shannon index;  $J$ , Pielou index;  $D$ , Simpson index.

**Table 4.** Diversity indexes of freshwater fungi species (Season and Lake).

| Season           | Shannon Index | Pielou Index | Simpson Index |
|------------------|---------------|--------------|---------------|
| Spring           | 1.95          | 3.57         | 0.77          |
| Summer           | 3.67          | 11.3         | 1.99          |
| Winter           | 3.55          | 9.84         | 1.9           |
| Lake             | Shannon Index | Pielou Index | Simpson Index |
| Dianchi Lake     | 2.65          | 5.79         | 1.15          |
| Fuxian Lake      | 1.72          | 2.77         | 0.64          |
| Xingyun Lake     | 2.4           | 4.26         | 1.04          |
| Yangzonghai Lake | 4.71          | 16.1         | 2.85          |

**Table 5.** Diversity indexes of freshwater fungi genera (Season and Lake).

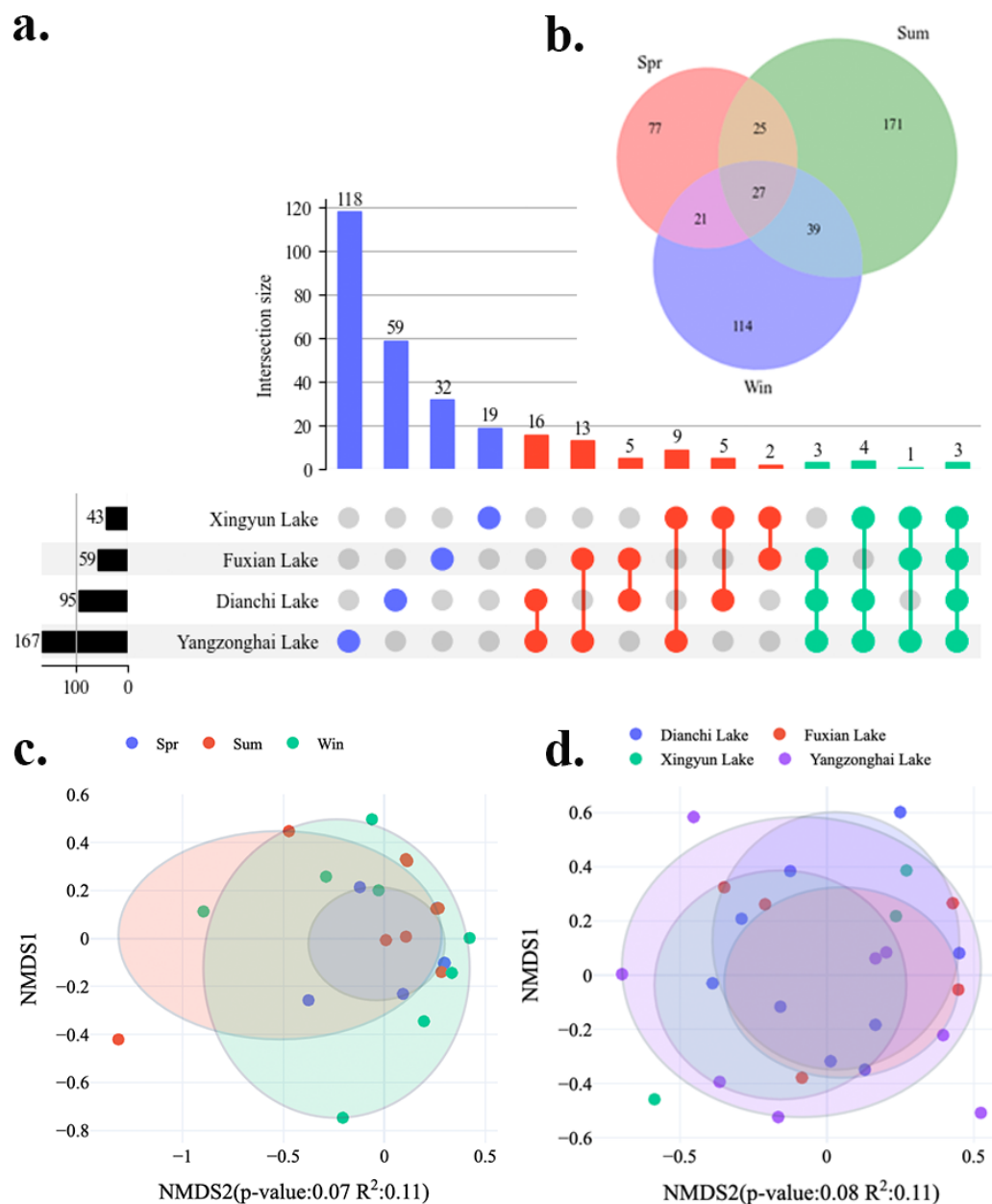
| Season           | Shannon Index | Pielou Index | Simpson Index |
|------------------|---------------|--------------|---------------|
| Spring           | 1.91          | 3.06         | 0.66          |
| Summer           | 3.61          | 9.33         | 1.64          |
| Winter           | 3.19          | 6.23         | 1.2           |
| Lake             | Shannon Index | Pielou Index | Simpson Index |
| Dianchi Lake     | 2.54          | 4.51         | 0.9           |
| Fuxian Lake      | 1.7           | 2.49         | 0.58          |
| Xingyun Lake     | 2.4           | 4.26         | 1.04          |
| Yangzonghai Lake | 4.51          | 11.36        | 2.01          |

At the genus level, the data were as follows—Shannon index: summer (3.61) > winter (3.19) > spring (1.91), Yangzonghai Lake (4.51) > Dianchi Lake (2.54) > Xingyun Lake (2.4) > Fuxian Lake (1.7); Pielou index: summer (9.33) > winter (6.23) > spring (3.06), Yangzonghai Lake (11.36) > Dianchi Lake (4.51) > Xingyun Lake (4.26) > Fuxian Lake (2.49); Simpson index: summer (1.64) > winter (1.2) > spring (0.66), Yangzonghai Lake (2.01) > Dianchi Lake (0.9) > Xingyun Lake (1.04) > Fuxian Lake (0.58).

Based on the distribution of fungi in different lakes, 118 species were exclusively found in Yangzonghai Lake, 59 species were exclusive to Dianchi Lake, 32 to Fuxian Lake, and 19 to Xingyun Lake. There were sixteen overlapping species between fungi in Yangzonghai Lake and Dianchi Lake; thirteen overlapping species between fungi in Yangzonghai Lake and Fuxian Lake; five overlapping species between fungi in Dianchi Lake and Fuxian Lake; nine overlapping species between fungi in Yangzonghai Lake and Xingyun Lake; five overlapping species between fungi in Dianchi Lake and Xingyun Lake; two overlapping species distributed in Fuxian Lake and Xingyun Lake. Three overlapping species were distributed in Yangzonghai Lake, Dianchi Lake, and Fuxian Lake; four overlapping species were distributed in Yangzonghai Lake, Dianchi Lake, and Xingyun Lake; and one overlapping species was distributed in Yangzonghai Lake, Fuxian Lake, and Xingyun Lake. Three overlapping species were distributed in four lakes *viz.* *Trichoderma* sp.10, *Fusarium* sp.1 and *Phaeoisaria* sp.2 (Figure 4a).

The Venn plot of fungi in different seasons showed that 77 species occur only in spring, 171 only in summer, and 114 only in winter. The intersection of spring and summer is 25, Spring and winter is 21, the intersection of winter and summer is 39, and the intersection of the three seasons (spring, summer, winter) is 27 (Figure 4b). The NMDS analysis indicated similar beta diversity among the four lakes, with Xingyun Lake being particularly similar to Fuxian Lake (Figure 4c). Furthermore, the diversity of fungi communities was also found to be similar across the lakes (Figure 4d). Lastly, the adonis analysis suggested that there were

no significant differences in the distribution patterns based on season ( $p > 0.05$ ,  $R^2 = 0.11$ ) and lake ( $p > 0.05$ ,  $R^2 = 0.11$ ).

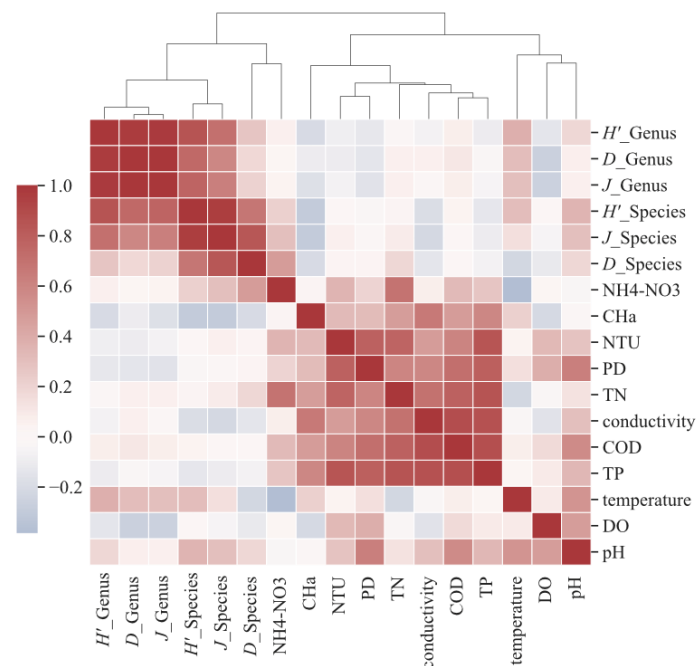


**Figure 4.** (a) The upset plot of fungi in different lakes; (b) The Venn plot of fungi in different seasons; (c,d) the NMDS analysis of area and season.

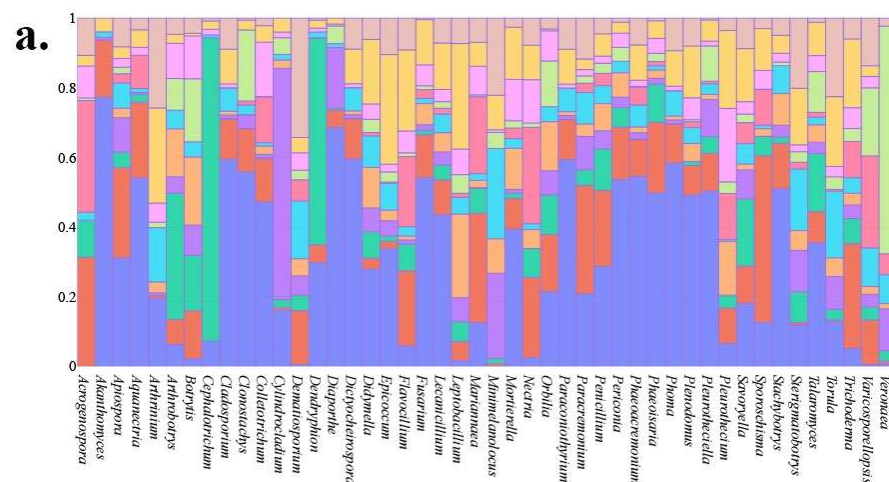
### 3.3. Correlation Analysis between Diversity Indices of Culturable Freshwater Fungi and Physical and Chemical Factors in Water Bodies

The Pearson correlation analysis, a statistical method, examined the relationship between physicochemical factors (Appendix A Table A1) and the diversity index of species and genus level in four lakes. This analysis indicated that the  $H'$ ,  $J$ , and  $D$  diversity index positively correlated with each in the genus and species group. This diversity index positively correlated with  $\text{NH}_4\text{-NO}_3$ , and the  $D$  index correlated highest with  $\text{NH}_4\text{-NO}_3$ . Meanwhile, the water properties of temperature, PH, and DO were correlated. The majority of physicochemical properties of water showed significant positive correlations. For instance, NTU exhibited highly significant positive correlations with TP, COD, TN, and

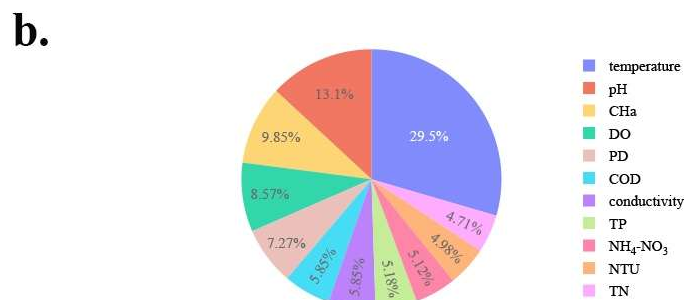
PD, while PD, TN, conductivity, COD, and TP demonstrated significant positive correlations. Notably, temperature and pH exhibited weak positive correlations with the indices belonging to genera and species ( $H'$ ,  $D$ ,  $J$ ), indicating that the diversity of fungi in water was influenced by pH and temperature (Figure 5). Furthermore, the random forest analysis, a machine learning algorithm, was used to identify the important environmental factors affecting genus distribution in four lakes (Figure 6a). The ranking analysis of various physicochemical factors showed that the contributions of temperature, pH, DO, and CHa were all greater than 8.5%. The cumulative contribution reached 61.02%, indicating that these four physicochemical factors mainly influenced the distribution of freshwater fungi in this study (Figure 6b).



**Figure 5.** The correlation plot of physical and chemical factors with the diversity index. CHa: Chlorophyll a; COD: Chemical oxygen demand; DO: Dissolved Oxygen; NTU: Nephelometric Turbidity Units; PD: Planktonic algae density; TN: Total nitrogen; TP: Total phosphorous; PH: *Pondus hydrogenii*.



**Figure 6.** Cont.



**Figure 6.** (a) The random forest analysis with physical and chemical factors to genera; (b) The ranking analysis of various physicochemical factors.

## 4. Discussion

### 4.1. Dominant Freshwater Fungal Communities in Four Lakes

The dominant genera of cultivable freshwater fungi in four lakes, Dianchi Lake, Fuxian Lake, Xingyun Lake, and Yangzonghai, are *Apiospora*, *Cladosporium*, *Clonostachys*, *Dictyocheiropora*, *Fusarium*, *Lecanicillium*, *Leptobacillium*, *Mariannaea*, *Phaeoacremonium*, *Phaeoisaria*, *Talaromyces*, and *Trichoderma*. The dominant species are *Trichoderma* sp.10, *Fusarium* sp.1, and *Phaeoisaria* sp.2.

*Phaeoisaria* predominantly occurs on leaves, barks, decaying wood, and twigs of plants from freshwater and terrestrial habitats. At the same time, some are isolated from surface marine sediments [55], some from soil [56], and some from saprobic decaying fruits [57]. Consequently, the habitats of *Phaeoisaria* are various. *Phaeoisaria* plays a significant role in nutrient and carbon cycling, the promotion of biological diversity, and ecosystem functioning of freshwater ecosystems, for their ability to decompose lignocellulose in the woody litter, softening the wood and releasing nutrients [11,58–60]. Nonetheless, some *Phaeoisaria* species are pathogenic to humans; for example, it has been reported that *Phaeoisaria clematidis* can cause corneal eye inflammation (keratitis) [61,62].

*Trichoderma* is commonly found in various lakes, indicating low nutritional requirements and can adapt to complex environments in plateau lakes. Moreover, *Trichoderma* can be isolated from soil, air, and plants, indicating its strong environmental adaptability and fecundity, which may be why it is widely distributed in various lakes. According to previous reports, it is known that *Trichoderma longibrachiatum* is used to produce xylanase [63]. *Trichoderma harzianum* is used to produce chitinase [64].

*Fusarium* species play diverse and significant roles in ecosystems, ranging from organic matter decomposition and nutrient cycling to interactions with plants and other microorganisms. Their activities influence ecosystem health, productivity, and stability, highlighting their importance in freshwater and terrestrial environments [65]. *Fusarium* species are diverse fungi with various applications across agriculture, industry, and biotechnology [66]. For example, *Fusarium solani* is known for producing various enzymes, including cellulases and xylanases, which are used in the textile, paper, and biofuel industries [67]. These enzymes help break down cellulose and hemicellulose, enhancing the efficiency of industrial processes [68].

However, *Penicillium* and *Aspergillus* were also identified in our study. *Penicillium* has been found in four lakes, while *Aspergillus* was only isolated in Yangzonghai. *Penicillium* was widely present in various lakes, with low nutritional requirements and strong adaptability to the environment. *Penicillium* species produce a range of secondary metabolites, including antibiotics (such as penicillin). These compounds can influence microbial community structure by inhibiting the growth of other microorganisms and shaping microbial interactions [69]. *Aspergillus* species play a crucial role in the decomposition of various organic materials, including plant residues and detritus. They facilitate the breakdown of complex polymers into simpler molecules that can be assimilated by other organisms. For example, *Aspergillus niger* is widely used in the production of industrial enzymes such

as amylases, pectinases, and cellulases, which find applications in industries like textiles, paper, food processing, and biofuel production [70].

#### 4.2. Diversity of Freshwater Fungal Species in Four Lakes

The findings of this study on microbial diversity in freshwater lakes, including species diversity, composition diversity, genetic diversity, ecological diversity, and functional diversity, are of significant importance [71]. Species diversity, in particular, is a crucial feature of microbial communities, reflecting the types and numbers of microorganisms in the same community and the differences in different communities [72]. The richness and relative evenness of microbial composition are also the results of ecological processes such as community environmental evolution, population invasion, diffusion, and competition [73].

A related study was conducted on the seasonal changes of culturable freshwater fungi in four lakes, and it was found that the number of culturable freshwater fungal isolates was highest in water bodies in summer, and all four lakes in this study were sampled in summer. This may be because the temperature in summer is suitable, the oxygen is sufficient, the enzyme activity of aquatic fungi and the ability to utilize organic matter in the water are high, and the reproduction rate is fast; it may also be that summer is the rainy season, and the water flow in the lake branches increases, which affects the surrounding rocks and soil. The strong scouring force of vegetation and vegetation allows microorganisms to be released from the surrounding environment into the lake water [74], resulting in a high diversity of freshwater fungi in the summer waters of the four lakes. From a geographical perspective, both Dianchi Lake and Yangzonghai are located in Kunming City, Yunnan Province. The total abundance of Dianchi Lake is 151, and Yangzonghai Lake is 280. The diversity index of species and genus showed that the freshwater fungal diversity in Yangzonghai is much higher than in Dianchi Lake. Fuxian Lake and Xingyun Lake are located in Yuxi City, Yunnan Province. The total abundance of Fuxian Lake is 74, while Xingyun Lake's is 60.

After analyzing our data, we observed a lack of significant seasonal and regional differences in fungal diversity. We speculate that several factors may contribute to this finding. First, many fungal species are highly adaptable and can thrive in a variety of conditions, resulting in a more uniform diversity across different seasons and regions. Generalist species may dominate, masking any potential differences. Additionally, our study site is primarily located in Yunnan Province, China, where the four lakes are situated in areas with relatively stable climates and habitats, likely leading to minimal seasonal or regional variations in fungal communities. Furthermore, fungal communities are influenced by complex interactions with other organisms, including plants, animals, and microbes. In some ecosystems, these interactions can buffer against significant changes in fungal diversity. Anthropogenic factors such as land use changes, pollution, and climate change may also alter fungal communities in ways that obscure natural patterns of diversity. Methodological factors may further impact our results. If sampling methods are not standardized or if there is insufficient replication, the observed lack of diversity might not accurately reflect true fungal diversity. For instance, collecting samples from similar types of habitats or at the same time of year may not capture the full range of fungal diversity. The sensitivity of detection methods also plays a role; traditional culture-based methods might miss rare or hard-to-culture species, while modern techniques like DNA sequencing, although more comprehensive, can still be influenced by factors like sequencing depth and primer selection. Moreover, if samples are collected infrequently or over a limited time frame, they may not adequately represent seasonal variability, as fungal diversity can change significantly throughout the year. Finally, the spatial resolution of sampling could affect our findings; regional diversity may appear uniform if sampling sites are too close together or if the sampling effort does not encompass a wide range of habitats within the region.

Moreover, 32 species were exclusive to Fuxian Lake and 19 to Xingyun Lake. However, diversity analysis found that the genus and species diversity of Xingyun Lake is higher

than that of Fuxian Lake. The pollution levels of the four lakes are also different. Dianchi Lake: IV, Fuxian Lake: II, Xingyun Lake: V, Yangzonghai Lake: II ([https://sthjt.yn.gov.cn/hjzl/hjzkgb/202306/t20230602\\_234194.html](https://sthjt.yn.gov.cn/hjzl/hjzkgb/202306/t20230602_234194.html), accessed on 1 May 2024). Yangzonghai and Fuxian Lake have relatively good water quality. Yangzonghai is also the lake with the highest diversity of freshwater fungi among the four lakes, consistent with previous research reports. The diversity and species richness of aquatic fungi in clean water bodies are higher than in polluted water bodies [75]. Xingyun Lake is the most heavily polluted lake among the four lakes. Although the total abundance is the lowest, the fungal diversity is not the lowest. The water quality of Fuxian Lake is the best, but the fungal diversity is the lowest among the four lakes. This may be related to the relatively low vegetation around Fuxian Lake. At the beginning of the study, it was found that there were fewer samples collected in Fuxian Lake. In summary, the pollution level of lakes can affect changes in fungal communities but have little impact on fungal diversity [76,77].

#### 4.3. Environmental Impact Factors of Freshwater Fungi in Four Lakes

The structure and diversity of freshwater fungal communities in plateau lakes—a complex and captivating subject of study—are influenced by a variety of factors. These include seasonal changes, temperature, pH, spatial distance, physical and chemical conditions, and water pollution.

For instance, pH, a key environmental factor in aquatic habitats, affects the diversity of aquatic fungi. Research shows that aquatic hyphomycetes generally prefer slightly acidic to neutral conditions. However, there is no clear linear correlation between pH and the overall abundance and diversity of aquatic fungi [7,33,78]. Nonetheless, the composition of freshwater fungal communities is closely related to pH.

Temperature is another crucial environmental factor. It influences not only the diversity and community composition of microorganisms but also their metabolic functions [79,80]. Although riparian vegetation, pollution, river conditions, and research methodologies can affect the diversity of freshwater lignicolous fungi, the general trend is that fungal diversity is higher in tropical and subtropical regions [58]. Studies indicate significant differences in fungal populations across subtropical, temperate, and tropical aquatic environments. Fungi from different climate zones exhibit varying optimal growth temperatures and levels of biological activity. Typically, the optimal growth temperature for fungi is between 20 and 25 °C, with tropical fungi thriving around 25 °C, but showing peak biological activity between 25 and 30 °C [81]. Thus, understanding the optimal growth temperatures of freshwater fungal populations and their peak biological activity provides valuable insights into how global climate change may impact these fungi and their response mechanisms.

Dissolved oxygen (DO) is a critical indicator of aquatic ecological health. Variations in DO levels affect microbial diversity and the enzymatic activity involved in organic matter decomposition. Research indicates that while DO levels are negatively correlated with microbial diversity, they are positively correlated with the activity of microorganisms involved in breaking down large organic molecules. High DO environments see increased dehydrogenase activity, whereas low DO conditions lead to higher activity of nitrogen and phosphorus cycling enzymes [82]. However, the impact of DO on aquatic fungi has been less studied.

Our research demonstrates that the total abundance of freshwater fungi is primarily influenced by temperature, pH, DO, and chlorophyll a (Chl a). This complex interplay of factors is especially evident in the four plateau lakes we studied, which are characterized by high latitudes and significant seasonal temperature variations. These findings suggest that seasonal changes can significantly alter microbial communities and water functions in these lakes.

## 5. Conclusions

For the first time, this study analyzed the diversity of culturable freshwater fungi in four representative lakes in Yunnan Province, China. Based on comprehensive analysis,

this study revealed a high species diversity of culturable freshwater fungi in four lakes of Yunnan Province, China. The number of culturable freshwater fungal isolates was highest in water bodies in summer. The significant differences in freshwater fungal species composition and distribution among these lakes are particularly noteworthy. The water environment factors, including temperature, PH, CHa, and DO, have emerged as key influencers, significantly shaping the distribution patterns of these fungi and, thereby, the overall freshwater fungal communities. However, the study has some limitations due to the use of culturable freshwater fungi. In future studies, it is very necessary to combine genomic analysis to study culturable and non-culturable fungi in lakes, so as to obtain more comprehensive and accurate information about the diversity of fungi in lakes.

**Author Contributions:** Conceptualization, L.L., H.G. and R.C.; methodology, L.L., Z.-X.Z., H.G., X.-A.W. and P.X.; software, L.L. and Z.-X.Z.; validation, L.L., Z.-X.Z., H.G. and S.C.K.; formal analysis, L.L. and Z.-X.Z.; investigation, L.L.; resources, L.L., X.-A.W. and P.X.; data curation, L.L., Z.-X.Z., X.-A.W. and P.X.; writing—original draft preparation, L.L.; writing—review and editing, L.L., Z.-X.Z., H.G., X.-A.W., P.X., S.C.K. and R.C.; visualization, L.L. and Z.-X.Z.; supervision, H.G. and R.C.; project administration, L.L. and R.C.; funding acquisition, L.L. and R.C. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study is supported by the Second Tibetan Plateau Scientific Expedition and Research (STEP) Program (Grant No. 2019QZKK0503).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

**Acknowledgments:** The authors would like to thank Qi Zhao for his generosity in providing the experimental platform and all the experiment costs, and Gao Chen and Junxing Yang and his research group for the water quality data of Fuxian Lake and Xingyun Lake. We also thank Peng Xing for the water quality data of Fuxian Lake and Yuwei Hu for his valuable modification comments. Samantha C. Karunarathna thanks the National Natural Science Foundation of China (32260004), Yunnan Revitalization Talents Support Plan (Young Talents and High-End Foreign Experts Programs), and the Key Laboratory of Yunnan Provincial Department of Education of the Deep-Time Evolution on Biodiversity from the Origin of the Pearl River for their support. We would like to thank Agrobiodiversity in Highland and Sustainable Utilization Research Group, Chiang Mai University, Thailand.

**Conflicts of Interest:** The authors declare no conflicts of interest.

Appendix A

Table A1. Physical and Chemical Parameters in Various Sites from Dianchi Lake, Fuxian Lake, Xingyun Lake and Yangzonghai Lake.

| Season | Lake             | CHa      | COD      | DO       | NH <sub>3</sub> –NH <sub>4</sub> | NTU      | PD         | TN       | TP       | Conductivity | pH       | Temperature |
|--------|------------------|----------|----------|----------|----------------------------------|----------|------------|----------|----------|--------------|----------|-------------|
| Spring | Dianchi Lake     | 0.007    | 5.76     | 7.22     | 0.27                             | 30.3     | 9,713,549  | 1.98     | 0.074    | 475.9        | 8.38     | 16.8        |
| Spring | Fuxian Lake      | 0.001    | 1.023333 | 7.9      | 0.025                            | 1.833333 | 514,407.7  | 0.143333 | 0.010667 | 317.8333     | 8.533333 | 16.4        |
| Spring | Xingyun Lake     | 0.003    | 7.076667 | 7.293333 | 0.035                            | 22.13333 | 18,186,113 | 1.223333 | 0.083333 | 589.8667     | 8.766667 | 18.46667    |
| Spring | Yangzonghai Lake | 0.004    | 4.88     | 9.21     | 0.025                            | 2.3      | 2,378,120  | 0.43     | 0.029    | 422.2        | 8.68     | 17.6        |
| Summer | Dianchi Lake     | 0.004    | 5.49     | 9.68     | 0.025                            | 40.7     | 38,632,520 | 0.89     | 0.075    | 404.7        | 9.13     | 25.5        |
| Summer | Fuxian Lake      | 0.003667 | 1.166667 | 7.39     | 0.025                            | 1.866667 | 710,880.7  | 0.153333 | 0.010667 | 310.1        | 8.593333 | 23.06667    |
| Summer | Xingyun Lake     | 0.012    | 6.72     | 7.21     | 0.025                            | 12.2     | 19,067,250 | 0.966667 | 0.074333 | 587.0667     | 8.853333 | 24.7        |
| Summer | Yangzonghai Lake | 0.002    | 4.9      | 7.6      | 0.025                            | 2.6      | 4,274,750  | 0.47     | 0.026    | 407.2        | 8.92     | 26          |
| Winter | Dianchi Lake     | 0.002    | 6.72     | 8.52     | 0.282                            | 16.3     | 30,521,660 | 1.29     | 0.052    | 433          | 9.1      | 18.7        |
| Winter | Fuxian Lake      | 0.00225  | 1.115    | 7.0075   | 0.025                            | 1.4      | 604,847.8  | 0.1375   | 0.00925  | 314.475      | 8.3725   | 19.25       |
| Winter | Xingyun Lake     | 0.00675  | 7.1175   | 7.5925   | 0.025                            | 22.3     | 39,477,183 | 1.27     | 0.0825   | 600.125      | 8.89     | 18.7        |
| Winter | Yangzonghai Lake | 0.003    | 4.4      | 7.44     | 0.025                            | 2        | 4,176,910  | 0.42     | 0.025    | 410.5        | 9.05     | 21.7        |

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